XYLITOL FROM OIL PALM EMPTY FRUIT BUNCH BY *CANDIDA GUILLIERMONDII*: FERMENTATION AND KINETIC STUDY

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By

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In the name of Allah, the Most Gracious and the Most Merciful

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LIST OF SYMBOLS

A, B or C	Coded factor	
A	Alpha (axial distance from centre point which makes the design rotatable)	
β_o	Constant coefficient	
β_i	Coefficient for linear effect	
β_{ii}	Coefficient for quadratic effect	
β_{ij}	Coefficient for cross-product effect	
Q_p	Xylitol productivities	g/L/h
Q_s	Substrate consumption	g//L/h
R^2	Coefficient of determination	
S_o	Initial concentration substrate	g/L
S_f	Final concentration substrate	g/L
t_o	Initial fermentation time	hr
t_f	Final fermentation time	hr
X_o	Initial biomass concentration	g/L
X_f	Final biomass concentration	g/L
Y	Response calculated by model (dependent variables)	
$Y_{p/s}$	Yield of product over the substrate consumption	g/g
$Y_{p/x}$	Yield of product over the biomass	g/g
$Y_{x/s}$	Yield of biomass over the substrate consumption	g/g

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
CaO	Calcium oxide
$Ca(OH)_2$	Calcium hydroxide
CCD	central composite design
CH_3COOH	Acetic acid
CO	Carbon monoxide
CPO	Crude palm oil
DoE	Design of Experiment
EFB	Empty fruit bunch
FFB	Fresh fruit bunch
FV	Final volume
g	gram
GC	Gas chromatography
hr	hour
HPLC	High performance liquid chromatography
IV	Initial volume
KOH	Kalium hydroxide
L	liter
NAD/NADH	Nicotinamide Adenine Dinucleotide
NaOH	Sodium hydroxide
NO_x	Nitrogen oxide
OH^-	Hydroxide ions
OPA	Oil palm ash
OPEFB	Oil palm empty fruit bunch

RI	Refractive index
rpm	rotation per minute
RSM	Response surface methodology
SD	Standard deviation
PM	Particulate matters
POME	Palm oil mill effluent
WHO	World health organization

XILITOL DARIPADA TANDAN KOSONG BUAH KELAPA SAWIT MENGGUNAKAN *CANDIDA GUILLIERMONDII*: FERMENTASI DAN KAJIAN KINETIK

ABSTRAK

Tandan buah kelapa sawit kosong adalah salah satu bahan buangan utama yang dihasilkan oleh kilang kelapa sawit. Bahan buangan yang mudah didapati, murah dan boleh diguna semula ini telah dipilih sebagai bahan asas untuk penghasilan xilitol, kerana ia mengandungi hemiselulosa, terutamanya xilan yang boleh dihidrolisis kepada xilosa. Oleh kerana kandungan xilitol dalam sumber semulajadi adalah sangat rendah, bahan yang mempunyai kadar harga yang rendah dengan kandungan xilosa yang tinggi telah dikenal pasti sebagai bahan pemula.

Analisis ke atas tandan buah kelapa sawit kosong menunjukkan terdapatnya 5 sebatian utama iaitu xilosa, glukosa, lignin, asid asitik dan furfural. Kepekatan sebatian tertinggi dikesan untuk xilosa pada 36.54 g/L. Ini menunjukkan tandan kosong buah kelapa sawit adalah bahan yang sesuai untuk penghasilan xilitol.

Dalam proses hidrolisis, penghasilan xilosa yang paling tinggi iaitu 28.97 g/L telah dikesan pada 120 ^{o}C, 15 minit masa tindakbalas dengan menggunakan 6% asid sulfurik. Proses hidrolisis telah dipanjangkan ke 300 minit untuk menentukan kehadiran bahan perencat seperti asid asitik dan furfural serta komponen gula yang lain iaitu glukosa. Di dalam proses peneutralan dan penyahtoksikan, kombinasi penggunaan kalsium oksida dan karbon teraktif menunjukkan kesan penurunan perencat yang tinggi, iaitu sehingga 69% kepekatan pada asid asetik dan 61 % kepekatan pada furfural.

Untuk proses fermentasi, *Candida guilliermondii* FTI 20037 telah dipilih sebagai biokatalis bagi penukaran xilosa kepada xilitol. Dengan menggunakan media sintetik, penghasilan xilitol tertinggi diperolehi di dalam Media 1, pada pH 3.5, 250 ppm, kepekatan substrat permulaan 250 g/L dan saiz inokulum 10 g/L. Penghasilan xilitol dengan menggunakan media ditoksifikasi dan media tidak-ditoksifikasi menunjukkan penghasilan xilitol per kepekatan xilosa ($Y_{p/s}$) ialah 0.73g/g dan 0.6 g/g, masing-masing. Ini menunjukkan media yang telah ditoksifikasi adalah substrat lebih baik berbanding media tidak-didetoksifikasi.

Penggunaan model tidak berstruktur bagi pertumbuhan, penghasilan xilitol dan penggunaan substrat telah dibandingkan. Didapati model Logistik bersesuaian dengan pertumbuhan *C. guilliermondii* dan penggunaan substrat, sementara model Leudeking-Piret pula adalah untuk penghasilan xilitol. Bagi perencatan substrat, model Andrew telah bersesuaian dengan pemerhatian eksperimen dengan R^2 =0.94. Proses pengoptimum telah dijalankan untuk mengenalpasti penghasilan xilitol yang tertinggi dengan menggunakan kaedah permukaan sambutan (RSM). Keadaan optimum bagi penghasilan xilitol telah dilihat pada pH 3.5, 250 rpm (kadar goncangan) dan saiz inokulum 10 g/L.

Xilitol telah dihablurkan pada -10 ^{o}C menggunakan kepekatan media sintetik dan media fermentasi yang berbeza. Proses penghabluran bagi kedua-dua media mengambil masa 4 jam dan 10 jam, masing-masing. Ini mungkin disebabkan kehadiran bahan asing di dalam media. Pada kepekatan xilitol yang tertinggi 597 g/L, 0.49 g/g, hablur xilitol telah diperolehi dengan 91.05% penulenan.

XYLITOL FROM OIL PALM EMPTY FRUIT BUNCH BY *CANDIDA GUILLIERMONDII*: FERMENTATION AND KINETIC STUDY

ABSTRACT

Oil palm empty fruit bunch (OPEFB) is one of the main wastes generated by palm oil mills. This waste is widespread, cheap and renewable and be selected as raw materials for xylitol production as it contains hemicellulose, comprising xylan that can be hydrolysed to xylose. As the xylitol content is very low in other natural sources, a low cost material with high content of xylose is identified as the starting material.

Analysis of oil palm empty fruit bunch fibers showed that there were 5 main compounds namely xylose, glucose, lignin, acetic acid and furfural. The highest compound concentration was detected for xylose at 36.54 g/L. This showed that an oil palm empty fruit bunch was a suitable material for the production of xylitol.

In hydrolysis process, the highest xylose production was detected at 120 ^{o}C, 15 min reaction time with 6% sulphuric acid at 28.97 g/L. The hydrolysis process was prolonged up to 300 min in order to determine the production of inhibitor components such as acetic acid and furfural, and also the presence of other sugar component such as glucose. In neutralization and detoxification process, the combination of calcium oxide and activated charcoal treatment showed the highest reduction of inhibitors, which was up to 69% on acetic acid and 61% on furfural concentration.

For fermentation process, *Candida guilliermondii* FTI 20037 was chosen as a biocatalyst for the conversion of xylose to xylitol. In synthetic media, the highest xylitol production was obtained in Media 1 at pH 3.5, 250 rpm, initial substrate concentration 250 g/L and inoculum size at 10 g/L. Production of xylitol using detoxified and non-detoxified media showed that the yield of xylitol per xylose concentration ($Y_{p/s}$) were 0.73g/g and 0.6 g/g, respectively. This showed that detoxified media was much better substrate compared to non-detoxified media.

An unstructured model taking into account growth, xylitol production and substrate consumption were compared. It was found that Logistic model fitted well with *C. guilliermondii* growth and substrate consumption, while Leudeking-piret model was for xylitol production. For substrate inhibition, Andrew model fitted well with the experimental observation with R^2 =0.94. The optimization process was performed to find out the optimum condition that lead to the highest production of xylitol using Random Surface Methodology (RSM). The optimum condition for xylitol production was observed at pH 3.5, 250 rpm agitation rate and 10 g/L of inoculum size.

Xylitol was crystallized at -10 ^{o}C using different concentration of synthetic and fermented media. The crystallization for both media took 4 hr and 10 hr, respectively. This could be due to the presence of other impurities in the media. At the highest xylitol concentration at 597 g/L, 0.49g/g xylitol crystal yield was obtained with 91.05% of crystal purity.

CHAPTER 1

INTRODUCTION

1.1 OIL PALM INDUSTRIES IN MALAYSIA

The Malaysian oil palm industries started in 1917 and grew slowly until 1950, when the agricultural diversification policy switches over from rubber to palm oil. Malaysia became the largest producer and exporter of palm oil, which then became a major agricultural industry in the country. In 2006, Malaysia produced about 51% of the world's palm oil and 62% of world export, which will expand more a head (Ahmad *et al.*, 2006).

Palm oil or *Elaeis guineensis* act as number one fruit crops in terms of production with 36.9 million tones for the year 2007 or 35.9% of the total edible oil in the world (MPOC, 2007). Malaysia's palm oil production will hit 20 million tones by 2020 (FORBES 2007). The oil prices have reached a new price of around RM 1800 to 1900/tonne (The Star, 2009). It was able to supply 12% of the global vegetable oil and 26% of the export trade in oils and fats (MPOC, 2006).

The total area under oil palm cultivation is over 2.65 million hectares, producing over 8 million tones of oil annually (Basiron, 2006). The oil consists of only 10% of the total biomass produced in the plantation. The remainder consists of a huge amount of lignocellulosic materials such as oil palm fronds, trunks and empty fruit bunches. According to Mahmudin and Puad (1999), the projection figures of these residues are 7.0 million tonnes of oil palm trunks, 26.2 million tonnes of oil palm fronds and 23% of empty fruit bunch (EFB) per tonnes of fresh fruit bunch (FFB) processed in oil palm mill.

An average oil palm mill can handle about 100 million tones of fresh fruit bunches daily. Figure 1.1 shows the extraction process in one of the palm oil mills. At the mills where oil extraction took place, solid and liquid wastes were generated. The solid residues, mainly EFB were more than 20% of the fresh fruit weight (Ma *et al.* 1993). For the liquid wastes, more than 500 kg (around 0.5 m^3) is produced, which is mainly in the form of palm oil mill effluent (POME), and are discharged during the processing of 1.0 million tones of fresh fruit bunches (Ma *et al.* 1996). Meaning that, 20 million tones of EFB and more than 50 m^3 of POME will be expected to be produced from a mill after processing 100 million tones of fresh fruit bunches.

The EFB is a lignocellulosic raw material and act as renewable resources, which is a valuable commodity in the market (MPOC 2007). Currently these fibers are used as boiler fuel and potassium fertilizer. The transformation of these fibers to a valuable food and pharmaceutical products is an advantage to the Malaysian economics. One of the valuable products that can be produced from these fibers are xylitol. Xylitol is a sugar alcohol, act as an artificial sweetener, which has dietary and high technological properties (Pepper and Olinger, 1988).

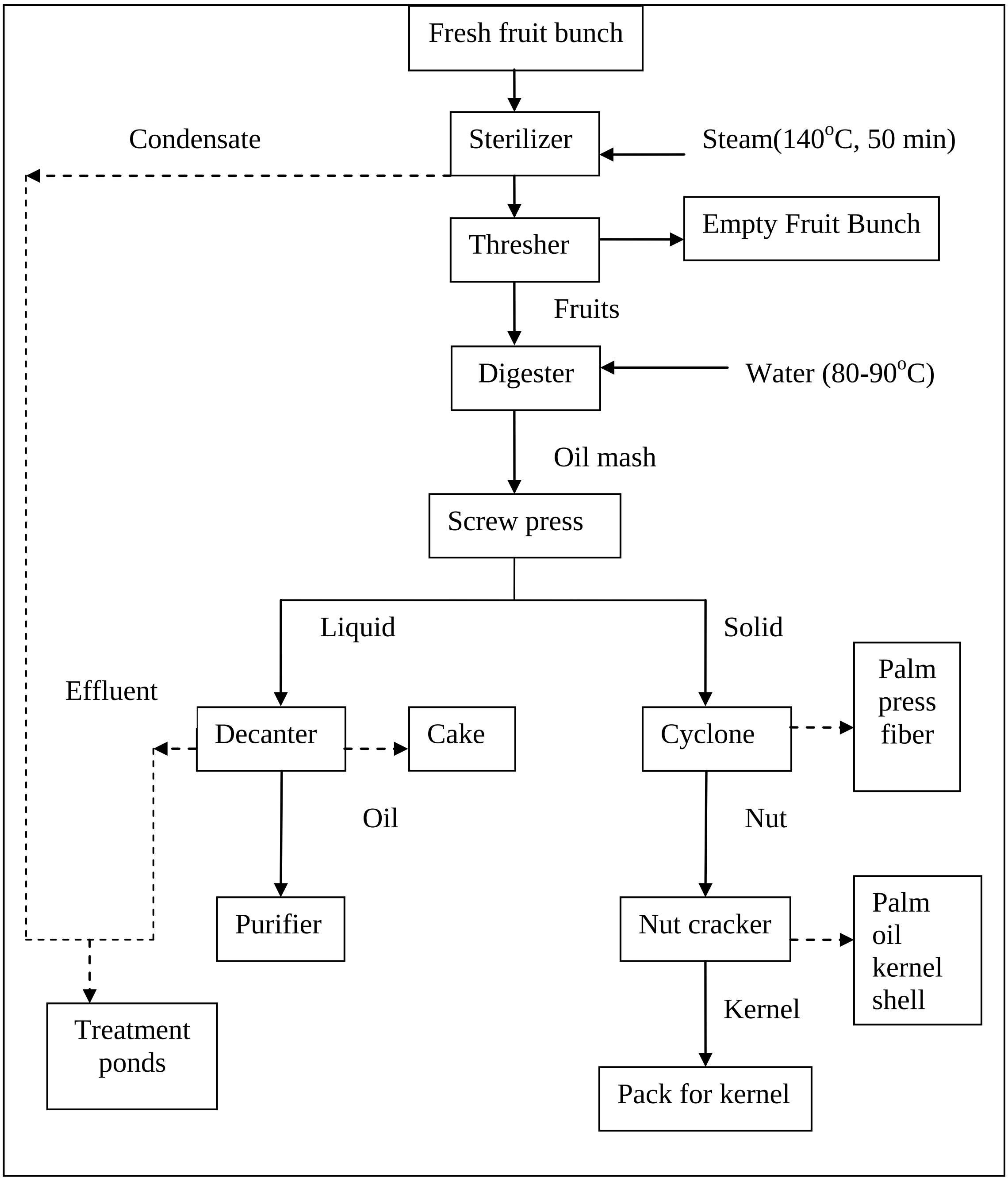


Figure 1.1 Palm oil milling process (_____)process (------) waste (MPOC, 2008)

Due to the need of industrial scale production and subsequent low down of the xylitol prices, EFB represents as a low-cost starting material for sugar production. In recent years, there has been an increasing trend towards more efficient utilization of agro industrial residues to xylitol such as sugarcane baggase (Marton *et al*., 2006), rice straw (Mussatto and Roberto, 2004), corn cobs (Dominguez *et al*.,

1997) and sorghum straw (Herera *et al.*, 2003) they provide a good substrate for microbial cultivation.

1.2 SUGAR INDUSTRY

Sugar production was established in the seventh century AD. Since then, the sugar industry has grown immensely. In 2008, Malaysia’s sugar production was only 84 thousand metric tons while the overall sugar consumption was 1,343 thousand metric tons (FAPRI, 2008). Increasing demand of sugar leads to imported sugar to meet the rising demand and compensate for the stagnant domestic production. For example, imports for the first 5 years of the 1990s averaged 885 000 tonnes per year, compared with 494 000 tonnes for the first-half of the 1980s, a 79 percent increase. In recent years, sugar has been Malaysia's largest agricultural imports, with annual sugar imports valued between US$200 to 300 million (FOA, 1997).

Sugar is an important source of food energy. During digestion, all food carbohydrates (starches and sugars) broke down into single molecule sugars. These sugars are absorbed from the intestine into the blood stream and travel to the cells, where they are used to provide energy for cellular functions. In parts of the world where people suffer from energy malnutrition and are undernourished, sugar is valued as an inexpensive source of energy to support human activities.

But regularly eating large amounts of sugar will cause serious harm. Sugar can cause hyperglycemia and weight gain, leading to diabetes and obesity in both children and adults. It leached the body vital minerals and vitamins and raised blood pressure, triglycerides, and the bad cholesterol (LDL), thus increasing the risk of heart disease. It causes tooth decay and periodontal disease, which leads to tooth loss and systemic infections. Besides that, it also make a child's difficult to learn, resulting in a lack of concentration. Both children and adults exhibit disruptive behavior, learning disorders, and forgetfulness from sugar consumption. It initiates auto-immune and immune deficiency disorders such as arthritis, allergies, and asthma. It also upsets hormonal balance and supports the growth of cancer cells (FAO, 1997). Therefore, the increasing amount of sugar in food, sweets and soft drinks could raised some concern about health effect. World Health Organization (2003) reported that the number of people suffer from diabetes, obesity, cancer and cardiovascular diseases are increasing every year especially in the developing world. In fact, about 1.2 million peoples in Malaysia are suffering from diabetes (The Star, 2006).

The Polyols sweetener industry is experiencing a rapid growth because of the increasing consumer demand for sugar-free and reduced calorie products (Mussatto *et al.*, 2006a). The sweeteners experiencing this rapid growth are the sugar alcohols such as xylitol, sorbitol, mannitol and maltitol. The name polyols refers to chemical compounds containing multiple hydroxyl groups. Sugar alcohols, a class of polyols, are commonly added to foods because of their lower caloric content than sugars; however they are also generally less sweet, and are often combined with high intensity sweeteners (Emodi, 1978).

Sugar alcohols are usually incompletely absorbed into the blood stream from the small intestines which generally results in a smaller change in blood glucose than sucrose. This property makes them become a popular sweetener among diabetics and people on low-carbohydrate diets. However, as for many other incompletely digestible substances (such as dietary fiber), over consumption of sugar alcohols can lead to bloating, diarrhea and flatulence because they are not absorbed in the small intestine. Some individuals experience such symptoms even in a single-serving quantity. With continuous use, most people develop a degree of tolerance to sugar alcohols and no longer experience these symptoms (Parajo *et al.*, 1998)

In Asia, xylitol is particularly in demand from the gum manufacturers; it was estimated that 80 to 90 per cent of chewing gum sold in the region now has the xylitol in their formulations. The China based company Futaste currently produces 35,000 tons of xylitol each year, as well as 20,000 tons of xylose and other types of sweeteners from corn cobs. The xylitol are now available in the market with the prices at RM 50 per kg (Charlotte, 2008). Hopefully with the development of biotechnological production of xylitol, the prices could be cheaper as consumer can use xylitol rather than other ordinary sweetener.

1.3 PROBLEM STATEMENT

Among various agriculture wastes in Malaysia, oil palm empty fruit bunch (EFB) is regarded as a promising agricultural resource as it is rich in cellulose and hemicellulose but is not effectively utilized. The industrial use of oil palm empty fruit bunch contributes to a reduction of an environmental pollution caused by their disposal and lost of potentially valuable resources. EFB on the other hand is known to give rise to deposits and corrosion problems. The hydrolysis of EFB to produce xylose solution can be a good alternative for this abandone product. This process has a double consequentces, i.e: the elimination of a waste and the production of a value-added product.

The industrial production of xylitol is based on the catalytic hydrogenation of xylose. This process requires the use of high pressure and temperature and extensive xylose purification steps, thus making a final product with a relatively high production cost. An alternative way to produce xylitol is by the biotechnological route, which can be more economically viable because it requires only a mild condition of temperature and pressure, and it eliminates the purification step since the microorganism specifically acts on xylose-to-xylitol bioconversion (Winkelhausen and Kusmanova, 1998). Microbial production of xylitol from agriculture wastes containing hemicellulose could be the best approach because it has a potential to realize cheaper production of xylitol with low environmental effect by effective utilization of renewable resources such as agricultural waste, oil palm empty fruit bunch (OPEFB) fibers.

1.4 RESEARCH OBJECTIVE

The main objective of this project is to study xylitol production by *C. guilliermondii* using oil palm empty fruit bunch hydrolysate as a substrate. The measurable objectives are:

1. to study the effect of multiple hydrolysis variable (reaction temperature, acid concentration and time) on the formation of xylose

2. to investigate the effect of various fermentation parameters for the production of xylitol by *C. guilliermondii* using synthetic and oil palm empty fruit bunch hydrolysate

3. to optimize fermentation variables for the production of xylitol in oil palm hydrolysate using Design of Experiment (DoE)

4. to compare kinetic models of microbial growth, xylitol production, xylose consumption and substrate inhibition by the tested yeast

5. To crystallize xylitol using synthetic and fermented media

1.5 ORGANIZATION OF THE THESIS

This thesis consists of five chapters that covers important details regarding this research.

Chapter One introduces the oil palm industries and the sugar industries in Malaysia. The problem statement is stated to give clear aims followed by the objectives of the study.

Chapter Two describes the fundamental characteristic of biomass residues and lignocellulosic materials. Explanations on hydrolysis process and neutralization and detoxification process were also discussed. In addition, the importance of xylitol production, fermentation process and xylitol recovery were also looked at. The last section discussed in details on the fermentation kinetics and Design of Experiments (DOE) for optimization and process parameters.

Chapter Three refers to the material and methods describing the experimental procedures of the present study. This chapter also covers the acid hydrolysis process, fermentation, kinetic and optimization studies, crystallization and analytical procedures.

Chapter Four discussed the experimental result together with the data analysis of various operating condition and process parameters. The detail explanations of the result are been divided into three main processes; hydrolysis, fermentation and crystallization. The kinetics and optimization of fermentation process using Response Surface Methodology have been discussed through this section. Each of the result will be followed by the discussion and comparison between the present results and the results obtained by others researchers.

Chapter five gives the overall conclusion based on the result obtained in Chapter 4. Recommendations for future research are also given in the chapter.

CHAPTER 2

LITERATURE REVIEW

2.1 OIL PALM RESIDUES

2.1.1 Processing and residues characteristic

Oil palm is a multipurpose plantation and also a prolific biomass producer which can be used as raw materials for value added industries (Basiron and Simeh, 2005). Fresh fruit bunch contains only 21% palm oil, while the rest are 6-7% palm kernel, 14-15% fiber, 6-7% shell and 23% empty fruit bunch (EFB) left as biomass (Umikalsom *et al.*, 1997). Oil palm residues (including shell, fibre and kernel) are cheap and abandoned materials produced during palm oil milling process (Figure 2.1). About 80% of these solid wastes were used as boiler fuel in many industries and 20% were left behind (Pansamut *et al.*, 2003).

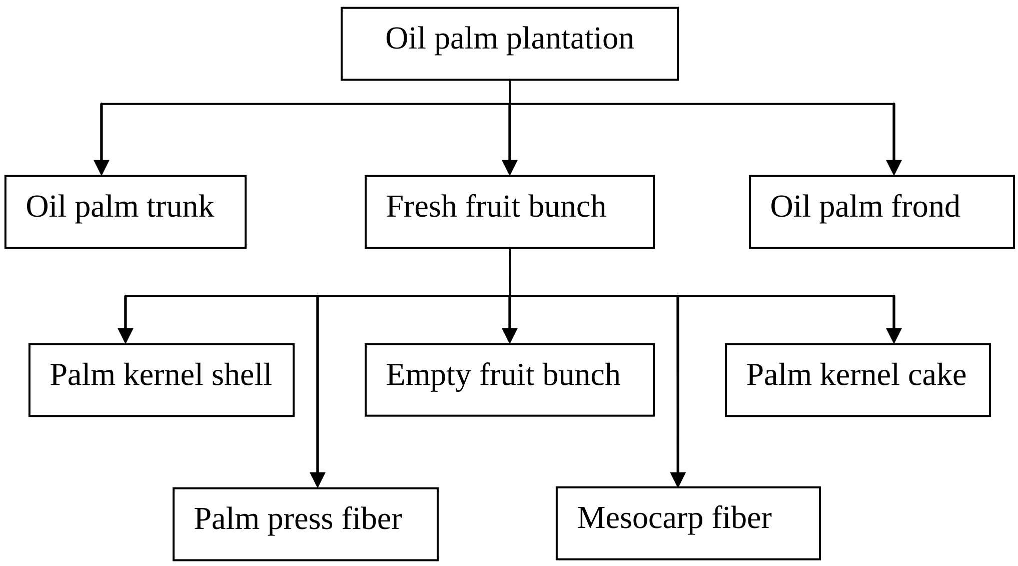


Figure 2.1 Palm oil residues generated from oil palm plantation (Sumathi *et al.*, 2008)

Oil palm empty fruit bunch (OPEFB) is generated as waste material by palm oil mills which consist of fibers. The fibrous materials were physically stick together to form vascular bundles. EFB from the mill contains 30.5% lignocellulose, 2.5% oil and 67% water. The main constituents of the lignocellulose were cellulose (45%), hemicellulose (32.8%) and lignin (20.5%). Of the hemicellulose, pentosan was 27.3%. It is estimated that oil palm empty fruit bunch comprises of 24% xylan, a sugar polymer made of pentose sugar (Muthurajah, 1981; Husin *et al.,* 1985; Ellis and Paszner, 1994).

Oil palm fiber is a non-hazardous biodegradable material extracted from oil palm empty fruit bunch (EFB) through decortication process. The fibers are clean, non-carcinogenic, free from pesticide and soft parenchyma cells. The empty fruit bunch is normally composed of a main stalk and numerous sharp and spines spikelets. The fresh sterilized EFB holds about 65% moisture, 30% dry matter and 2-5% crude palm oil (Husin *et al.,* 2002).

Oil palm fronds are available daily throughout the year when the palms were pruned during the harvesting of fresh fruit bunch. Oil palm trunk is obtained during the replanting of the oil palm trees. EFB, mesocarp fiber and shell were collected during the pressing of sterilized fruits (Gurmit *et al.,* 1999).

2.1.2 Oil palm residues in environmental matrices: general consideration

The oil palm industries produced more than one hundred million tonnes of residues worldwide. One hectare of oil palm plantation generates about 21.62 tonnes per year of biomass residues. Fronds and EFB were the most residues produced which was 50.31% and 20.44%, respectively (Singh *et al*., 1999; Saka, 2005; Goyal *et al*., 2006).

The utilization of biomass residues from palm oil by-products (fiber & shell) as fuel source were identified as the main cause of the emission released. Economically, the usage of palm residues as fuel is seen as productive as this material will not be wasted. However the combustion process in the furnace of the boiler released the emission of particulate matters (PM), carbon monoxide (CO), nitrogen oxide (NO_x) and sulphur dioxide (SO_x). Due to that, monitoring and control of these pollutants from the palm oil mill is of great concern to the community (Prasertsan and Prasertan, 1996).

The residues generated at the palm oil mills such as empty fruit bunches, fibres and kernel shells can be used to produce electricity to supply the national grid. Technologies are now available to harness the biogas from effluent ponds of the mills for power generation. If the biogas is fully exploited, more of the fibre and kernel shell can be alternatively used for power generation to supply the national grid or for composite fibreboard production. In the future, the carbon credit derived from the use of biogas and biomass for power generation may contribute further revenue to the country. For example, methane (biogas) fetches USD 10/tonne of tradable carbon under the Kyoto Protocol when it came into effect in the year 2008 (Basiron and Simeh, 2005).

MPOB research has also shown that palm oil is a good source for liquid fuel. It has been established that CPO can be burnt as fuel. During the 2001 downturn in palm oil prices, it was also shown that CPO can be blended with medium fuel oil to be used as fuel for boilers. Up to 6518 tons of CPO was burnt as fuel in the national utilities power plant at Prai, Pulau Pinang. The bigger potential is in respect of conversion of palm oil into methyl esters (palm diesel). Road trials involving buses have shown that it can be used as a diesel substitute with positive environmental effects. The palm diesel plant can also produced beta carotenes and vitamin E that can contribute to the viability of the biodiesel project. The other option that MPOB research has unfolded is the possibility of blending processed palm oil with diesel (5%-10%). Malaysia is currently in the process of preparing the regulatory framework to enable the government to mandate the use of biofuel when the situation warrants such an intervention (MPOC, 2008).

2.1.3 Oil palm residues and potential utilization

The supply of oil palm residues from the oil palm and palm oil processing by-products is seven times the availability of natural timber. Each year, the oil palm industries in Malaysia generate more than 30 million tonnes of residues in the forms of empty fruit bunches, oil palm trunks and oil palm fronds. Palm residues such as empty fruit bunches and trunks are being used for commercial products (*e.g.* pulp and paper, medium density fibreboard, automotive components *etc*.). Effective utilization of palm residues into value-added products has the potential to generate another RM 20 billion in the next 10-15 years (Basiron and Simeh, 2005).

Currently, oil palm residues are converted into various types of value added products via several conversion technologies that are readily available. For example, fibers from EFB were found to be an ideal material for the making of mattresses, seats, insulation and etc (Basiron and Simeh, 2005). EFB has been investigated as a raw material for building materials, solid fuel pellets, chemical products, particleboard, fiberboards, blockboard, and pulp and paper (Muthurajah, 1981; Kobayashi *et al.*,1985; Husin *et al.*, 1985; Gabriele, 1995).

The latest researches by MPOB onto EFB are to convert EFB into paper-making pulp. The pulp is then bleached using the total chlorine-free (TCF) methods to obtain sheets of paper. Pulp and paper from oil palm biomass can be used in many ways such as cigarette paper and bond paper for writing (Gurmit *et al.*, 1999; MPOC 2006).

Palm fibers are versatile and stable and can be processed into various dimensional grades to suit specific applications such as mattress cushion production, erosion control, soil stabilization/compaction, landscaping and horticulture, ceramic and brick manufacturing, thermoplastic filler, flat board manufacturing, paper production, acoustics control, livestock care, compost, fertilizer and animal feed (Sumathi *et al.*, 2008).

Oil Palm Ash (OPA) produced from incinerating the empty fruit bunch were used as fertilizer due to its high organic and nutrient content beneficial to crop. In the other cases, fibers, shell and empty fruit bunches were used as a source of energy for the processing mill itself to generate heat and electricity (Yusoff, 2006).

The research by local scientist proved that palm kernel, EFB, palm shells and stones can be converted to oil palm activated carbon (Jia and Aik, 2002; Jia and Aik, 2000). The oil palm activated carbon has been used to treat toxic air such as carbon monoxide (CO) and SO_x (Sumathi *et al.*,2008).

The oil palm trunk is converted to various types of wood such as saw-wood and ply-wood or lumber. Oil palm lumber has been successfully utilized as core residues in the production of blackboard. The sawn-wood produced from oil palm can be used to make furniture but not for building structure due to its low specific density. Oil palm trunk also has been used to produce particleboard with chemical binders. Some of the trunks were mixed with EFB and oil palm press fiber to be combusted and produced energy (Gurmit *et al.*, 1999).

Oil palm fronds were also a source of food for ruminants (cattle and goats). Fronds were left to rot in between the rows of oil palm trees in the plantation for soil conservation, increased the fertility of the soil, increased the amount of water retained in the soil, erosion control and provide a source of nutrient to the growing oil palm trees (nutrients is recycled as a long term benefits) (Husin *et al.*, 2002).

Palm oil mill effluent (POME), a high volume liquid waste which are non toxic has been used for cellulase production. Cellulase was identified as one of the key enzyme degrading cellulose (Kotchoni *et al*., 2003). It finds extensive application in food, fermentation and textile industries (Muthuvelayudham and Viruthagiri, 2006).

Oil palm biomass can also be potential feedstock raw materials to chemical and biochemical industry. The cellulose component can be hydrolyzed to yield glucose from which ethanol, citric acid, butanol and other single cell protein can be obtained through chemical and microbiological transformation. Hemicelluloses, also present in the biomass can yield pentoses especially xylose which upon hydrolysis can be converted to xylitol, furfural, furan, resins and furfuryl alcohol. The lignin fraction of oil palm biomass is a potential source of phenolic resins (Basiron and Simeh, 2005).

However, these renewable materials can be alternatively used for producing valuable chemical products (fuel, and chemical feedstock) by applying the thermochemical conversion process, including pyrolysis, liquefaction and gasification as well as supercritical fluid extraction methods (Yaman, 2004).

2.2 LIGNOCELLULOSIC MATERIALS

Lignocellulosic material is an abundant and inexpensive source of sugar which can be microbiologically converted to industrial products such as fuel alcohol, chemical and protein for food and feed purpose. Lignocellulosic biomass, such as corn stover, wheat bran, sugar cane baggase and oil palm empty fruit bunch were easily available and produced in a large scale in the agriculture developing country. It can be directly or indirectly used for the production of biomolecules and commodity chemicals. However, some of these applications were limited by the close association exist among the three main components in lignocellulosic: cellulose, hemicellulose and lignin. Therefore clear understandings of the chemistry towards identifying the reason why it was so resilient to biological process such as hydrolysis and fermentation is urgently needed (Ramos, 2003).

2.2.1 Cellulose

Cellulose is a straight chain polymer that consists of glucose unit linked together by β (1-4) glycosidic bonds (Figure 2.2). It is an insoluble molecule consisting of 2000 - 14000 residues. The size of cellulose molecule is given in terms of its degree of polymerisation. However conformational analysis of cellulose indicated that cellobiose is the basic structural unit rather than glucose (Ramos, 2003).

Cellulose is found in large amounts in nearly all plants, and is potentially a major food source. The cellulose chain bristles with polar -OH groups. These groups form many hydrogen bonds with OH groups on adjacent chains, bundling the chains together. The chains also pack regularly in places to form hard, stable crystalline regions that give the bundled chains even more stability and strength. The effect of the bonding by hydrogen bond increased the rigidity of cellulose and causes the cellulose highly insoluble in most solvent (Jeoh, 1998).

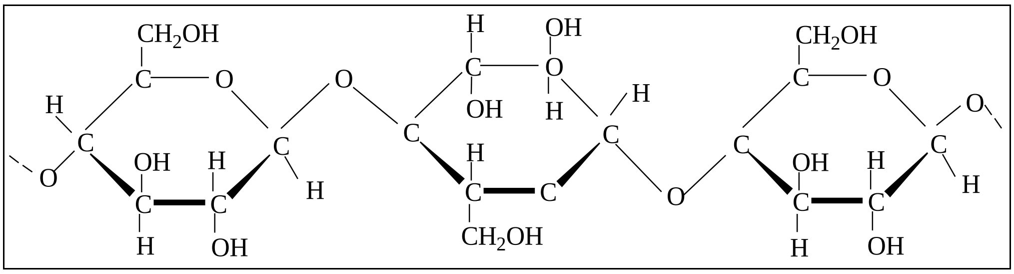


Figure 2.2 Structure of cellulose (Xiang *et al.*, 2004)

2.2.2 Hemicellulose

Hemicellulose is the second most abundant natural polysaccharides after cellulose. It comprises of one fourth to one third of most plant materials and this amount will vary according to the particular plant species. Hemicelluloses are linear polymers composed of cyclic 5-carbon and 6-carbon sugars (polysaccharides). They are mainly composed of pentose (xylose, rhamnose and arabinose) and hexose (glucose, mannose and galactose) sugars which can be reduced to monomeric sugars primarily to xylose and glucose by hydrolysis with mineral acid (Saha, 2003).

Hemicellulose is considerably easier to hydrolyze than cellulose. Studies have shown that hydrolysis of hardwood hemicellulose at 120-140 ^{o}C using 2.5% H_2S0_4 gave xylose yields exceeding 80% in most cases (Kim and Lee, 1987).

Many studies utilized the hemicellulose portion of agriculture residues like *Eucalyptus grandis* (Silva *et al*., 1998a), rice straw (Roberto *et al*., 1994), aspen wood hemicellulosic hydrolyzate (Preziosi-Belloy *et al*., 2000), barley bran (Cruz *et al*., 2000), hybrid polar wood chips (Dominguez *et al*., 1997), and corn cobs (Rivas *et al*., 2003) for xylitol production.

The hemicellulose content of softwoods and hardwood differ significantly (Fengel and Wegener, 1989). Hardwood hemicellulose mainly composed of highly acetylated heteroxylan, generally classified as para-o-methyl glucoronoxylan. Hexosans were also present but only in small amount (Ramos, 2003). In contrast, softwoods have two principal hemicelluloses: galacto-glucomannan (70% mannan), which made up approximately 60% of the total hemicellulose content, and arabino-4-0-methylglucuronoxylan (65% xylan), which constitutes the remaining 40%. The amount of galactose/mannan and arabinan/xylan was used to estimate the quantities of the major and minor hemicellulose component of the wall (Timell, 1967; Highley, 1987). Softwood hemicellulose contained a high proportion of mannose units and more galactose unit than hardwood hemicellulose whereas hardwood hemicellulose contained a high proportion of pentose (Fengel and Wegener, 1989).

Xylan is a major constituent of hemicellulose (Figure 2.3). It is a polysaccharide that can be hydrolyzed into D-xylose, which is also known as wood sugar. Xylan consists of about 200 β-xylopyranose residues, linked together by 1, 4-glycosidic bonds. However they contain smaller propartions of uronic acid, but are more highly branched and contain large proportion of L-arabinofuranosyl unit (Saha, 2003).

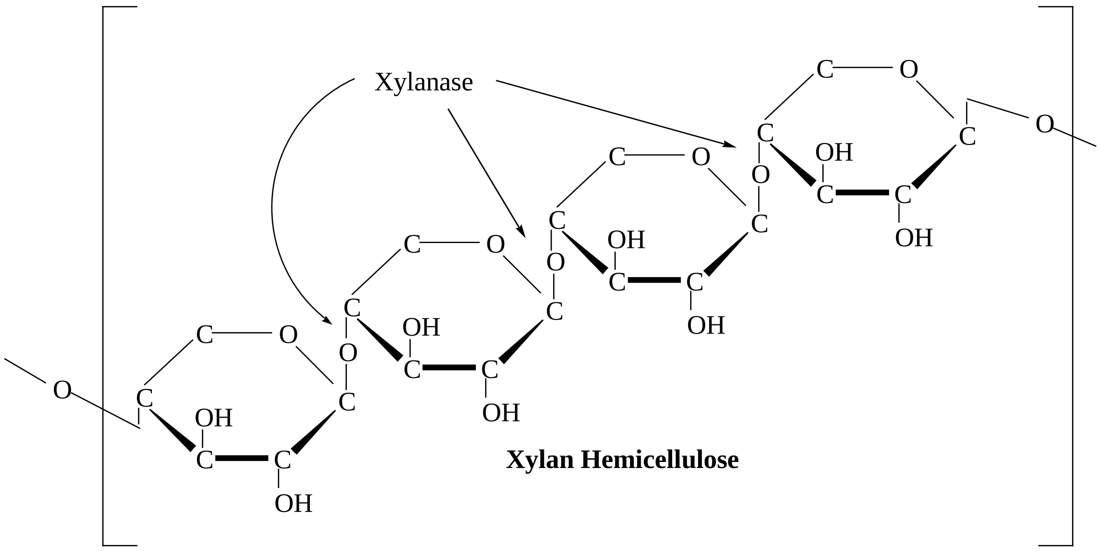
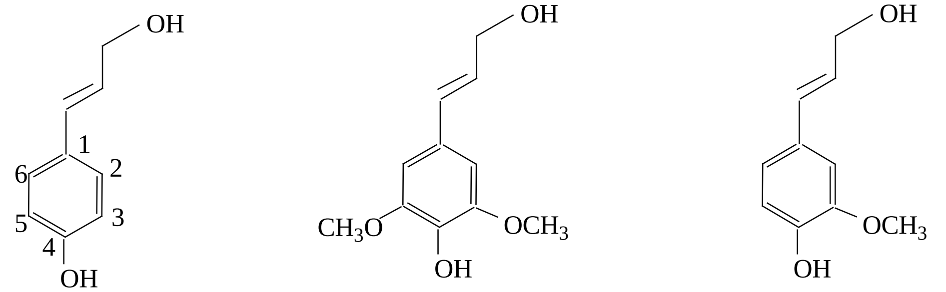


Figure 2.3 Structure of Xylan Hemicellulose (Sigma Aldrich, 2008)

2.2.3 Lignin

In plant tissues, hemicelluloses are generally combined with lignin (Fengel and Wegener, 1989). Lignin is a three dimensional polymer of aromatic compounds covalently linked with xylan in hardwood and galactoglucomannan in softwoods

(Garg *et al.,* 2007). Its structure composed of phenylpropane monomer namely para-coumaryl alcohol, coniferly alcohol and sinaply alcohol, which were generally referred as cinnamyl alcohol, and were commonly called lignin C9-units (Figure 2.4). It contributes to approximately 15% to 35% of the dry mass of softwoods, hardwoods and woody grasses. Lignin is deposited between individual wood fibers and act as an intercellular adhesive, binding individual wood fibers together. Lignin was usually insoluble in all solvents, unless it is degraded by physical or chemical treatments (Ramos, 2003).



p-coumaryl alcohol   Sinapyl alcohol   Coniferyl alcohol

Figure 2.4 Structures of lignin monomer (Deacon, 1997)

2.3 HYDROLYSIS OF LIGNOCELLULOSIC MATERIALS

Hydrolysis is a process of splitting compound into fragment by the addition of water. In acid hydrolysis process, acid is used as a catalyst in the splitting process. The hydrolysis process in dilute acid medium is very complex, as the substrate is in a solid phase and the catalyst in a liquid phase. Dilute acids leads to a limited hydrolysis, this occurs in the hydrolysis of hemicellulosic fraction, leaving the cellulose and lignin fraction almost unaltered (Karimi *et al.,* 2006)

Dilute acid hydrolysis appeared to be in the best position from the economic viewpoint (Wyman, 1994). Therefore using selected operational condition, it is possible to hydrolyze almost quantitatively hemicelluloses leaving the cellulose and lignin in the solid residue, which can be processed for the conversion of glucose solution to ethanol through fermentation process (Parajo *et al.*, 1995) and the production of paper pulp (Grethlein and Converse, 1991).

Sulphuric acid (Nguyen *et al*, 2000), hydrochloric acid (Springer, 1966), or acetic acid (Conner and Lorenz, 1986) are acids commonly employed as catalysts. These acids released protons that break the heterocyclic ether bonds between the sugar monomers in the polymeric chains formed by the hemicelluloses and cellulose. The breaking of these bonds released several compounds, mainly sugars such as xylose, glucose and arabinose. Other compounds released are oligomers, furfural and acetic acid. A quantitative hydrolysis of the hemicelluloses can be performed almost without damage to the cellulose because the bonds in hemicelluloses are weaker than in cellulose (Bungay, 1992).

In dilute-acid hydrolysis the hemicellulose fraction is depolymerized at lower temperatures than the cellulose fraction. If higher temperatures (or longer residence times) were applied, the formed monosaccharide from the hemicellulose will degrade (Saeman, 1945), which gave rise to furan compounds and carboxylic acids (Taherzadeh *et al*., 1997; Larsson *et al*., 1999).

The degradation of hemicellulose is a gradual process during acid hydrolysis treatment in which long polymers are gradually degraded to oligosaccharides and finally monosaccharide. The oligosaccharides are rather short-lived (Lee *et al*., 1999), and very often they are not analyzed. The liquid phase, containing the monosaccharide, is removed between the treatments, thereby avoiding degradation of the monosaccharide formed. Avoiding degradation of monosaccharide is important, not only to improve the yield, but also to avoid inhibition problems, since the degradation products are toxic to the fermenting microorganisms (Taherzadeh *et al*., 2000; Larsson *et al*., 1999).

Acid hydrolysis process was developed to use less severe condition to achieve high xylan to xylose conversion yields. Achieving high xylan to xylose conversion yield is necessary to achieve favorable overall process economics because xylan accounts for up to a third of the total carbohydrate in many lignocellulosic materials. It was also reported that the amount of sugar released during hydrolysis depend on the type of raw material and operating condition of the experiment. As a consequence, the amount of sugars recovered from the raw material is dependent on the reaction time, temperature and acid concentration (Pessoa *et al*., 1996). According to Neureiter *et al*., (2002), acid concentration was the most important parameter affecting sugar yield, while for the formation of sugar degradation products, temperature had the highest impact. Normally, dilute acid hydrolysis was carried out at temperature between 120 ^{o}C and 200 ^{o}C.

2.3.1 Inhibitors

Hydrolysis process of hemicellulosic fraction produced pentose sugar mainly xylose and arabinose. However, this also results in the formation of inhibitory degradation by-products such as acetic acid, furfural and hydroxylmethylfurfural.

The amount and nature of inhibiting compounds depend on the raw material, the hydrolysis procedure and the reaction time in the hydrolysis process (Olsson and Hahn-Hagerdal, 1996).

Leonard and Hajny (1945) reported that there are four classes of inhibitors which are:

1. minerals and metals that contain in lignocellulosic materials or resulting from the corrosion of the hydrolysis equipment
2. product derived from the hydrolysis of hemicellulose such as acetic acid, furfural and hydroxymethylfurfural
3. product derived from lignin degradation such as phenolic compound, aromatic acid and aldehydes and
4. compounds derived from extractives such as vanillic, syringic, caproic and palmitic acid

Inhibitors contained in lignocellulose hydrolyzates could limit the consumption of the carbon source, reduced the growth kinetic, or even hinder the fermentation process. Occasionally, inhibition was a result of synergistic effects. The direct neutralization of neutralized hydrolyzates (without further processing) usually reduced the efficiency of fermentation, both the growth and the product formation being affected (Frazer and McCaskey, 1989; Olsson and Hahn-Hagerdal, 1996).

Acetic acid (CH_3COOH) was released from the hydrolysis of the acetyl group in the hemicellulose, as a consequence of deacetylation of acylated pentosan (Taherzadeh, 1999). Since acid was not further hydrolyzed, the formation of acetic acid depends on temperature and pressure of dilute acid hydrolysis until the acetyl group was fully hydrolyzed. Its inhibitory action depends on the concentration of the undissociated form, which was a function of both concentration and pH. At acidic pH, acetic acid can diffuses into cell cytoplasm, where it dissociates and lower the intracellular pH, resulting in uncoupled energy production and impaired transport of various nutrients with increased ATP requirement. Acetic acid interference results in an increased in the ATP required for this maintenance function, as well as interferes with the cell morphology. Lawford and Rousseau (1998) reported that acetic acid toxicity is related to the ability of undissociated (protonated) weak acid (pKa= 4.75) to transverse the cell membrane and to act as a membrane protonophore, which causes acidification of the cytoplasm.

Based on previous study on the bioconversion of xylose to xylitol, employing *C. guilliermondii* FTI20037 cultivated in semi synthetic medium revealed that the acetic acid concentration determined its degree to toxicity, since a concentration as low as 1.0 g/L favoured the bioconversion, while the concentration higher than 3.0 g/L inhibited xylose consumption and xylitol formation. Studying the inhibitory effect of acetic acid (6 g/L) using a synthetic medium containing xylose, and found that the xylitol yield was 0.66 g/g with a volumetric productivity of 0.38 g/L/h (Felipe *et al.,* 1995).

Furfural and hydroxymethylfurfural were also released in the hydrolyzate from hexose degradation causing delayed of the fermentation process or ultimate death of the organism (Figure 2.5). Furfural has been shown to reduce the specific